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An Assessment of Fecal Water Contamination in the Absaroka Beartooth Wilderness

By

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Thesis

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Abstract – Abstract – Wilderness water sources are often defined as pristine or high quality due to the lack of point source pollution. Non-point source pollution from recreation to water resources can be extensive and is well-studied in protected areas globally. Bacterial contamination, specifically fecal bacteria, poses a significant threat to human health because of the risk for outbreaks of illness and disease. Water sources in designated Wilderness areas are particularly vulnerable to fecal water contamination due to high volume of backpackers and lack of backcountry waste facilities. To estimate the occurrence of fecal water contamination in Wilderness water resources, an exploratory analysis was conducted at various lakes across the Absaroka Beartooth Wilderness, MT/WY using fecal indicator bacterium (FIB), total coliform/*Escherichia coli*. At sites that tested positive for the FIB, microbial source tracking (MST) via ddPCR was conducted using a human-specific *Bacteroides* marker (BacHu) and a universal *Bacteroides* marker (AllBac) to determine the contribution of humans to total *Bacteroides* contamination in water sources. Results of our analysis reveal the occurrence of fecal indicator bacteria and evidence of human fecal contamination at a number of sites. In addition, management interventions such as setbacks may not be having their desired or intended effect on protecting water quality. To protect Wilderness Character and water quality managers must take additional steps to prevent fecal water contamination in Wilderness areas while maintaining a rigorous aquatic monitoring program.

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## Thesis

This thesis will constitute three chapters. Chapter one will define the issue in question (fecal contamination of Wilderness water resources), highlight motivations to address the issue, and identify methods to better understand the issue while formulating questions and hypotheses. Chapter two will describe my process in applying a study design to a specific area to gather and analyze data to help better understand the state of the issue. In chapter three I will test my hypotheses and attempt to answer my questions while reflecting on the process as well as providing suggestions and outlook for future studies of this nature.

# Chapter 1

## Introduction

Wilderness water sources are frequently characterized as high quality due to the lack of anthropogenic disturbances within the designated boundaries (Montana Department of Environmental Quality). In the state of Montana, waters within the boundaries of designated Wilderness areas are considered outstanding water resources due to the restrictions on new or increased point source pollution sources (Horwich 1993).

The conundrum for Wilderness areas is that most of the water pollution comes in the form of non-point source or diffuse pollution such as run off from camping areas (Cilimburg et al., 2000)(Figure 1). Although permanent development and human occupation is restricted in designated Wilderness (Zahniser 1964), short-lived recreational activities can degrade water quality (Cilimburg et al., 2019). Water degradation can be derived from contaminants associated from campsites and subsequent non-point source runoff to Wilderness water resources (Marion et al., 2016)

Of the many visitor contaminants that may enter water sources, fecal water contamination is an looming public health issue due to the potential transmission risk of illness and disease (Apollo et al 2017). Restrictions on permanent facilities to manage human waste may make Wilderness areas vulnerable to fecal water contamination due to open defecation by visitors in high congestion areas. Wilderness managers must identify potential hazardous water sources by determining the occurrence of fecal bacteria and the source of fecal water contamination to protect Wilderness resources and



subsequent Wilderness character.

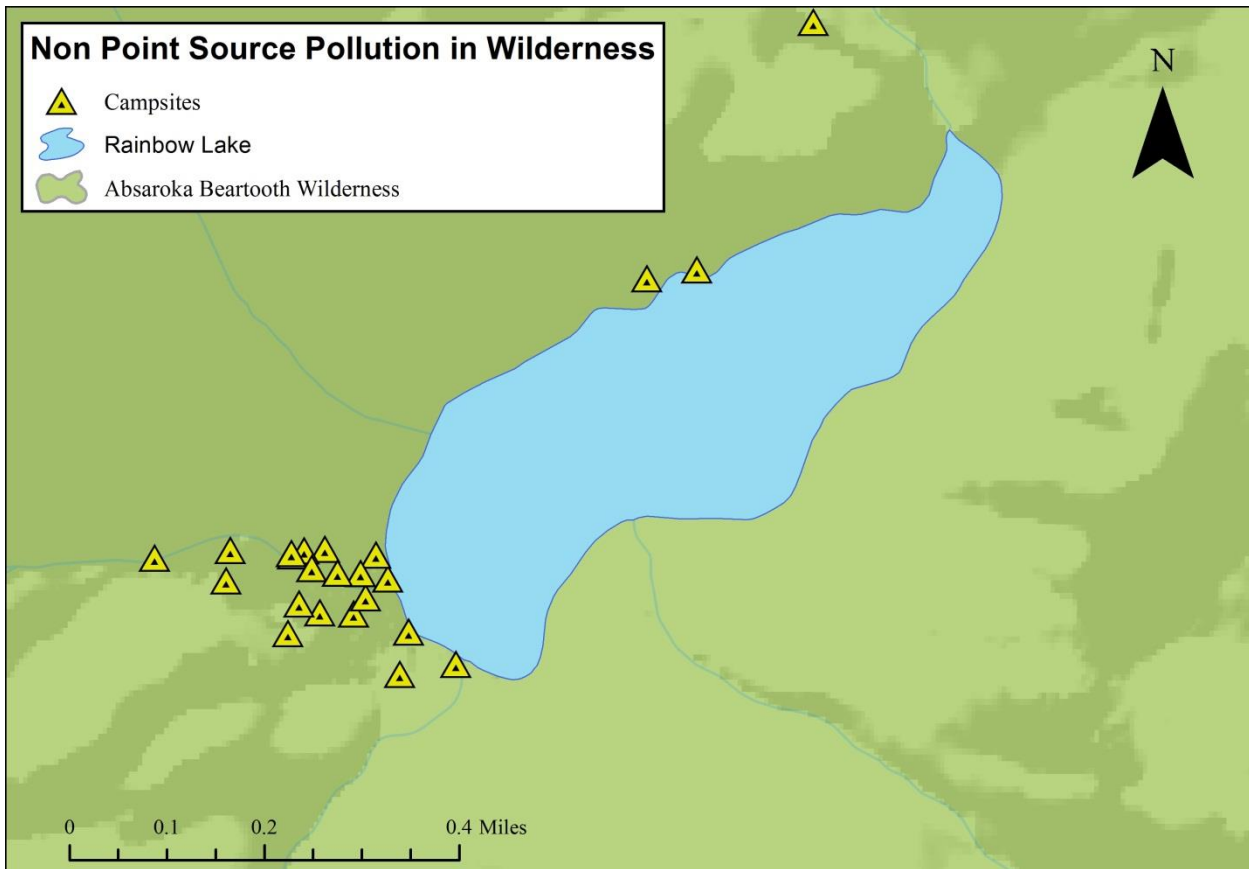


Figure 1- An example of an area of potential non-point source pollution in Wilderness

## Wilderness Background

Protected areas around the globe are often created based on the benefits they provide to society (IUCN 2009). In the United States, the impetus for creating our first protected areas was rooted in public health in response to the ill effects of industrialization and resource exploitation (NPS 2011). The creation of public lands in the US is frequently referred to as the democratization of clean air, clean water, and outdoor recreation (NPS 2013). Our public land system in the US provides visitors a natural contrast to their modern lives in more urban areas; away from pollution, congestion, and other symptoms of industrialization.

Officially instituted in 1964, the National Wilderness Preservation System is the federal system of managing designated Wilderness that has been adopted by several land management agencies including the Forest Service (USFS), Bureau of Land Management (BLM) and the National Park System (NPS) (Landres et al. 2008). Designated Wilderness is often referred to as “capital ‘W’ Wilderness”, because the use of the word “wilderness” dates back hundreds of years, long before the creation of US federal land agencies (Nash 2014). The difference between capital “W” and lower case “w” wilderness are the formal restrictions placed on anthropogenic activities (Cole 1989).

Designated ‘Wilderness’ areas in the United States are lauded globally for their natural condition due to the strict restrictions in place on anthropogenic activities (Driver et al 1987). According to International Union for the Conservation of Nature (IUCN) Wilderness areas are ranked 1b, second highest on their international protected area list, a ranking system roughly based on the extent to which anthropogenic activities are restricted in a particular protected area (Dudley 2008).

According to the Wilderness Act (Zahniser 1964), within Wilderness areas,

“...there shall be no commercial enterprise and no permanent road within any wilderness area designated by this Act and except as necessary to meet minimum requirements for the administration of the area for the purpose of this Act (including measures required in emergencies involving the health and safety of persons within the area), there shall be no temporary road, no use of motor vehicles, motorized equipment or motorboats, no landing of aircraft, no other form of mechanical transport, and no structure or installation within any such area.”

While many natural areas may provide the aesthetic condition and natural resources we associate with “wildness”, Wilderness is a specific land designation carrying stricter regulations that make it unique among other land designations (Cole 1989). What sets Wilderness apart from other areas are the restrictions on roads, permanent structures, as well as on motorized and mechanized equipment (Zahniser 1964). These restrictions are in place to protect overall quality of Wilderness experiences often called Wilderness character.

## Wilderness Character and Monitoring

Wilderness areas have many restrictions on human activities to protect resources and provide impetus for preserving what known as Wilderness character (Landres et al. 2005).

The criteria composing Wilderness character are defined in section 2(c) as;

....(1) generally appears to have been affected primarily by the forces of nature, with the imprint of man's work substantially unnoticeable; (2) has outstanding opportunities for solitude or a primitive and unconfined type of recreation; (3) has at least five thousand acres of land or is of sufficient size as to make practicable its preservation and use in an unimpaired condition; and (4) may also contain ecological, geological, or other features of scientific, educational, scenic, or historical value.

According to section 2 (b) of the Wilderness act, agencies are required to monitor and preserve Wilderness character (Landres et al. 2008). While the Wilderness act has no specific directions for preserving Wilderness character, legal and Wilderness scholars often refer to section 2(c), the definition of Wilderness, to understand congressional or legal intent for the meaning of Wilderness character (Rohlf and Honnold 1988, McCloskey 1999, Scott 2002).

Over the years agencies and their outside partners have developed specific protocols for monitoring impacts to Wilderness character (Landres et al. 2008). Finally, in 2005, a general framework was formalized by the agencies and their partners for the monitoring of impacts to selected conditions related to Wilderness character derived from section 2(c) (Landres et al. 2005). The framework identifies five qualities related to Wilderness character that guide the selection of conditions to monitor; 1) Untrammeled, 2) Natural, 3) Undeveloped, 4) Outstanding opportunities for solitude or a primitive and unconfined type of recreation, 5) The unique qualities of a certain wilderness area (Landres et al. 2008).

While the framework provides general guidance for monitoring Wilderness Character, Wilderness managers must adapt the framework to select relevant impacts to monitor that are specific to the conditions of the Wilderness area in their jurisdiction (Landres et al. 2005). Subsequently, US

Wilderness areas are in wide range of biogeographic areas from Florida to Alaska (Tricker and Landres 2018) and the type and scale of impacts to Wilderness character may vary substantially from Wilderness area to Wilderness area depending on the ecosystem and the type of use (Marion et al., 2018). To monitor Wilderness character effectively, managers must identify specific impacts from recreational use that are relevant their area (Landres et al. 2005). These impacts usually stem from primitive recreational activities like backpacking, boating, and outfitting, since permanent developments and motorized/mechanized equipment are restricted (Cole and Landres 1996).

## **Recreational Impacts to Wilderness**

Although visitors are restricted to primitive forms of recreation, recreational impacts in Wilderness areas can be extensive and are well studied in the field of recreation ecology (Cole 1989; Leung et al. 2001; Monz et al. 2010). Recreation ecology is the science of assessing the impacts of recreation to natural resources such as impacts to clean air, clean water, wildlife, and aesthetics, among others, in and outside of protected areas (Cole 1989). Because different forms of recreation have varying impacts on natural resources, managers must monitor impacts that are specific to the popular types of recreation as well as the type of ecosystem in their jurisdiction (Leung and Marion 2000).

Common among Wilderness areas are the backcountry opportunities they offer for overnight recreation (Cole 1990). Backpackers and horse packers are drawn to Wilderness for the chance to escape civilization for days or even weeks at a time. The remote nature of Wilderness areas and the restrictions on backcountry facilities also require users to manage their own waste (Apollo 2017). This lack of managerial oversight often leads to extensive impacts from overnight use at areas experiencing high congestion of overnight visitors (Cole 1990, Cilimburg et al. 2000; Derlet and Carlson 2004, 2006).

Campsites are the major location of impact in Wilderness areas because they are the main places where people congregate and concentrate their impacts (Marion and Cole 1996; Cole and Monz 2004; Marion et al. 2018). Some of the major impacts stemming from campsites are soil erosion, loss of native vegetation, introduction of invasive species, trash accumulation, and accumulation of human waste (Marion and Cole 1996; Cole and Monz 2004). In addition to the impacts on the terrestrial environment, campsites are often located near water sources such as lakes, springs and streams creating a risk for the contamination of aquatic systems used for cooking, cleaning, and drinking water (Cilimburg et al. 2000; Apollo 2017).

Recreational impacts to aquatic systems are extensive and are well studied in protected areas across the globe (DeLuca et al. 1998; Derlet and Carlson 2006; Reed and Rasnake 2016) and may include sedimentation, nutrient enrichment, loss of aquatic species, and bacterial contamination (Marion et al. 2016). While recreational impacts can directly and indirectly affect aquatic ecosystem function over time, bacterial contamination poses the most imminent threat to water quality and human health due to the risk of the transmission of fecal borne illness and disease (Cilimburg et al. 2000).

## **Fecal Water Contamination**

Fecal contamination in water resources is widely studied across the globe from public drinking water systems to rural agricultural catchments, high elevation villages, and Wilderness areas. (Pope et al. 2003; Farnleitner et al. 2010; Tambalo et al. 2011; Paruch et al. 2015; Forester and Scott 2016). Pathogenic bacteria, like coliforms and *Bacteroides*, can persist in the intestines of warm-blooded mammals as well as in the natural environment and are used globally as designated standard fecal indicator bacteria (FIB) in aquatic and terrestrial ecosystems (Farnleitner et al 2010; Paruch et al 2015). There are three overlapping groups of coliform bacteria: total coliform, fecal coliform, and *Escherichia coli*. While total coliform bacteria originate from several different sources in the

environment, occurrence of total coliform can indicate general fecal contamination (APHA 2005). Testing for total coliform/*E.coli* is an efficient way to determine whether a water resource is a public health hazard since testing for individual pathogens is costly and time consuming (Derlet and Carlson 2006).

Certain pathogenic bacteria (e.g. *E. coli*) are some of the most successful lifeforms on earth due to their ability to adapt to environmental perturbation (Ishii et al., 2007). Although *E. coli* mostly resides in the intestines of warm-blooded animals, its facultative nature and ability to attach to surfaces and utilize nutrients allows total coliform/*E.coli* to persist for long periods of time in the open environment (Chekabab et al., 2013). For example, in the Bridger Mountains of Montana (USA), evidence of *E. coli* occurrence in the environment was still present a year after first entering the terrestrial environment, unaffected by depth of fecal burial (Temple et al. 1982). Furthermore, when *E.coli* enters the aquatic environment, some strains have been shown to persist for weeks to over ten months (Warburton et al., 1998; McGee et al., 2002).

Fecal water borne pathogenic bacteria such as *E. coli* are vectors for disease worldwide (Chekabab et al.2013; Apollo 2017). Although not all strains of *E. coli* are harmful to humans, many strains may cause illness (e.g. infectious diarrhea, pneumonia) and certain strains of *E. coli* (e.g. *E.coli* 0157:H7) have resulted in multiple human fatalities (Chekabab et al. 2013). Detection of total coliform/*E. coli* in surface waters may be a sign of fecal contamination and if detected, the sources of contamination should be identified to protect water quality ergo public health (Pope et al 2003). Past researchers have identified *E. coli* contamination in a variety of water sources in Wilderness areas and subsequent potential human health issues (Clow et al. 2013; Forrester and Scott 2016).

## **Wilderness Human Waste Management and Health Implications**

Wilderness areas have similar issues to those of underdeveloped regions across the globe due to the lack of modern waste management facilities. Bacterial contamination to water sources is a major problem internationally especially in rural areas (i.e., livestock production areas, remote towns, mountain villages) and developing countries (i.e., nations of Africa and SE Asia) (Bain et al 2014). Illness caused by the consumption of fecal contaminated water is a major cause of mortality in the developing world (Prüss-Ustün et al. 2014). Sources of fecal contamination can include faulty sewer systems, leaking septic tanks, and presence of wildlife or open defecation (Cilimburg et al. 2000; Ahmed et al. 2012; Spears et al. 2013).

Restrictions on facilities to contain human waste challenge efforts to maintain high water quality in protected areas therefore Wilderness areas are vulnerable to fecal contamination due to open defecation by recreationists (Apollo 2017). Although some recreationalists may choose to pack out their waste, most do not and either openly defecate or bury their excrement (Apollo 2016). Although it is suggested to bury human waste (LNT 2019), once fecal matter leaves the host and enters the terrestrial environment, harmful fecal associated bacteria such as *E.coli* and *Salmonella* can persist for over a year regardless of burial depth (Temple et al 1983).

Although water resources are used for some recreational activities (e.g. fishing, swimming) in Wilderness, water for drinking, cleaning, and cooking are the most ubiquitous uses (Cilimburg et al 2000). Under EPA standards for drinking water, if *E.coli* is detected (i.e. > 1 colony forming unit per 100 ml of water) in a particular drinking water source, it is deemed a public health hazard (APHA 2005). Wilderness water studies conducted in the Sierra Nevada and the Smoky Mountains suggest that total coliform/*E.coli* are most likely to occur in water sources adjacent to areas with high backcountry recreational use (Clow et al.2013).

US land managers are becoming increasingly aware of the accumulation of human waste in water sources in Wilderness and other areas (Cilimburg et al. 2000; Derlet and Carlson 2004; Marion et al.

2018). Studies across the globe have shown high occurrence of fecal contamination in the water sources of protected areas experiencing high recreational use (Derlet and Carlson 2004; Clow et al. 2013; Forrester and Scott 2016). In the United States, popular hiking trails such as the Pacific Crest Trail and the Appalachian Trail have observed occurrence of fecal coliforms in many popular water sources in repeated sampling (Derlet and Carlson 2006, Clow et al. 2011, Reed and Rasnake 2016). The growing number of visitors to these trails coupled with the limited resources of land agencies challenges Wilderness managers seeking to maintain acceptable natural conditions or Wilderness character (Landres et al 2008).

Popular backcountry areas in Denali National Park, on the John Muir Trail, and Mount Everest each experienced outbreaks of diarrhea due to fecal water contamination (McLaughlin et al 2005; Meyer et al 2017; Khan et al 2018). The areas of contamination were identified as popular water sources in areas congestion such as base camps for popular mountain summits and other popular camping areas (Apollo 2016). At Denali National Park, medical researchers interviewed 132 climbers where 29% of respondents reported experiencing diarrhea at some point on the way to summit Denali in the summer of 2002. The study suggests that open defecation in high congestion areas is the main cause of fecal contamination recreational water sources (McLaughlin et al 2005).

A similar study conducted along the John Muir Trail in the Sierra Nevada mountain range in California showed that of 737 valid respondents, 16% had experienced diarrhea on their backpacking trip (Meyer et al 2017). On Mount Everest at a popular basecamp, 62 of 126 personnel (49%) contracted traveler's diarrhea on their attempt to summit the world's highest peak even with properly trained guides and required sanitation equipment (Khan et al 2018.) Authors in these studies highlight the dangers associated with open defecation and subsequent fecal contamination in popular water sources and the implications for public health (McLaughlin et al 2005; Meyer et al 2017; Khan et al 2018).



## **Wilderness Management Interventions for Protecting Water Quality**

Managers often rely on education as an intervention to prevent human waste accumulation, through mechanisms such as informational signs, trail ambassadors, and educational programs such as Leave No Trace (LNT) to encourage users to properly dispose of their waste (Marion and Reed 2007). Education is an inexpensive way to encourage proper waste disposal (e.g. packing it out or digging a cathole) and is widely utilized by managers across the Wilderness management system (LNT 2019). Education is a popular tool for Wilderness managers to prevent improper waste disposal, but the effectiveness of such “cognitive” fixes (i.e., relying on public education to inspire behavior change) has been questioned by social psychologists (Heberlein 2012).

In addition to education, agencies may also choose to institute zoning restrictions around waterbodies, often called ‘setbacks,’ for high impact recreational activities such as cooking, cleaning, and camping (Marion et al. 2018). Many of these restrictions align with LNT suggestions for low impact camping. According to LNT, Wilderness users should cook, clean, camp, and dispose of human waste at least 100 ft from streams and 200 ft from lakes (LNT 2019). These suggestions have been instituted as formal Wilderness regulations in some areas to prevent the impacts of recreation on natural resources like water sources (Marion et al. 2018). Whether or not setbacks prevent water quality impacts such as bacterial contamination is unknown. Further, setbacks may not be effective without adequate enforcement; a challenge for Wilderness managers with little resources.

The enforcement of restrictions such as burial and setbacks is necessary to ensure that the restrictions are followed by recreationists (Cole 1996). Wilderness rangers are the main enforcement mechanism in Wilderness areas and carry the authority to write violation notices (VNs) to visitors disobeying Wilderness regulations (Roggenbuck and Berrier 1981). Effective enforcement of

Wilderness regulations presents a challenge for agencies due to the limited number of Wilderness rangers and the vast areas they oversee in their jurisdictions (Cole 1996).

With a limited number of Wilderness rangers and resources, managers need be creative in targeting their management strategies in an effective manner (Landres et al. 2008). Monitoring efforts such as Wilderness Character data collection provide managers with information on highly impacted areas which can help efficiently direct their enforcement and regulatory efforts (Landres et al 2005). Parameters from Wilderness character monitoring data provide measures of impact such as mineral soil lost and percent vegetation lost (called Barren Core) which is informative for identifying sites experiencing high recreational impact (Wilderness Institute 2013).

Currently there are no specific guidelines for monitoring impacts to water quality in the Wilderness. Although some studies have looked at the impacts of recreation on water quality, there is no established aquatic protocol under Wilderness character monitoring (Marion et al .2017). Methods and results from recreation ecology studies provide a template for Wilderness managers to develop a recreational impact monitoring protocol for water quality to address impacts to the aquatic resources affecting the “natural” character of Wilderness.

## **Methods for Measuring Fecal Water Contamination**

The standard method for detecting fecal contamination in water often is based the on detection of fecal indicator bacteria such as fecal coliforms to indicate fecal contamination (APHA 2005). Testing for total coliform/*E.coli* in surface waters can be relatively simple and inexpensive for agencies (Derlet and Carson 2004). Water from the source of interest is collected in a sealed polypropylene container on site, and then transported to a lab within 48 hours for analysis (Pope et al 2003). The samples are then filtered and the filters are incubated in a proprietary broth for 24 hours, enumerated, and analyzed for quantification of coliforms/*E.coli* in colony forming units (CFUS)

(Millipore 2019). The short holding time may make it difficult to transport samples from remote areas but the quick analysis gives managers results within 72 hours of sampling (Pope et al 2003; Millipore 2019).

Although total coliform/*E.coli* is proven fecal bacterial indicator and insightful about possible pathogens in the water, it is non-host specific; the host species cannot be determined without a separate molecular analysis (Botes et al 2013; Paruch et al 2018). Since total coliform/*E.coli* resides in the intestines of most endothermic animals (e.g. birds, ungulates, humans) it is only partially insightful in determining the source of fecal contamination (Savageau et al 1983; Ishii et al. 2008; Tambalo et al 2011).

To determine whether or not fecal contamination is coming from humans requires genetic tools that rely on genetic markers to adequately identify the host species (Tambalo et al 2011; Paruch et al 2015). An emerging technology called microbial source tracking (MST) is revolutionizing the way scientists think about sources of fecal contamination (Scott et al 2002). Using molecular methods like environmental DNA (eDNA) analysis and polymerase chain reaction (PCR), researchers can accurately identify the host associated with particular bacteria (EPA 2005).

PCR has rapidly evolved over the past 15 years from a novel technology to a mainstream management tool (Paruch et al 2015). This MST method uses host specific markers or ‘primers’ to attach to existing identical DNA strands in any sample for new DNA synthesis of the target primer (Delidow et al 1993). First, genetic primers and florescent probes are selected for the targeting of specific organisms in a particular sample. Next, DNA is extracted in preparation for the PCR reaction and combined the primers and probes (Tambalo et al 2011; Paruch et al 2015). The role of the primer is attaching onto identical target strands of the DNA of interest with matching florescent probes to highlight the reaction if the target DNA is present in the sample.

PCR is based on three simple steps required for any DNA synthesis reaction: (1) denaturation of the target DNA into single strands; (2) annealing of primers to each original strand for new strand synthesis; and (3) extension of the new DNA strands from the primers for synthesis of DNA. These reactions result in the synthesis of defined portions of the original DNA sequence. If the reactions are successful, the researcher has successfully replicated the target DNA according to the genetic primer and will be able to quantify the reaction via counting the fluorescence emitted from the probes (Delidow et al. 1993).

For MST of fecal contamination in water sources, researchers have developed host specific primers for the fecal bacteria genus *Bacteroides*, a common gut pathogenic bacterium in humans and animals (Paruch et al 2015). Unlike the facultative *E.coli* which has low host specificity, is temporally variable and can replicate in the environment (EPA 2005; Field & Samadpour 2007; Farnleitner et al. 2010, Paruch et al 2015), *Bacteroides* represent a large group of highly host specific, anaerobic bacteria making it an ideal candidate for microbial source tracking of recent host specific fecal contamination ((Bernhard & Field 2000; Hold et al. 2002; Dick et al. 2005; Paruch et al 2015). *Bacteroides* is becoming the standard target for MST and a number of host specific markers for *Bacteroides ssp.* have been developed including humans, horse, ruminants, swine, among others (Dick et al. 2005; Layton et al. 2006; Reischer et al. 2007; Shanks et al. 2008; Lamendella et al. 2009, Tambalo et al 2011; Paruch 2015).

## **Absaroka Beartooth Wilderness Management**

The Absaroka-Beartooth Wilderness (ABW) is located on the Custer-Gallatin National Forest and includes the largest contiguous alpine plateau in the United States (Farmer and Cook 2013). This expansive alpine Wilderness is a popular attraction for visitors due to its proximity to Yellowstone National Park and for its extensive backcountry trail network (USFS 2011). Home to hundreds of

alpine lakes and extensive stream networks that provide drinking water to thousands of visitors annually (Watson 2014), the ABW provides a potential baseline to understand potential public health hazards stemming from recreational impacts. While many people assume that water sources in Wilderness areas are of high quality, but research has shown a risk of fecal contamination by recreationists (Derlet and Carlson 2004; Johnson and Spildie 2014).

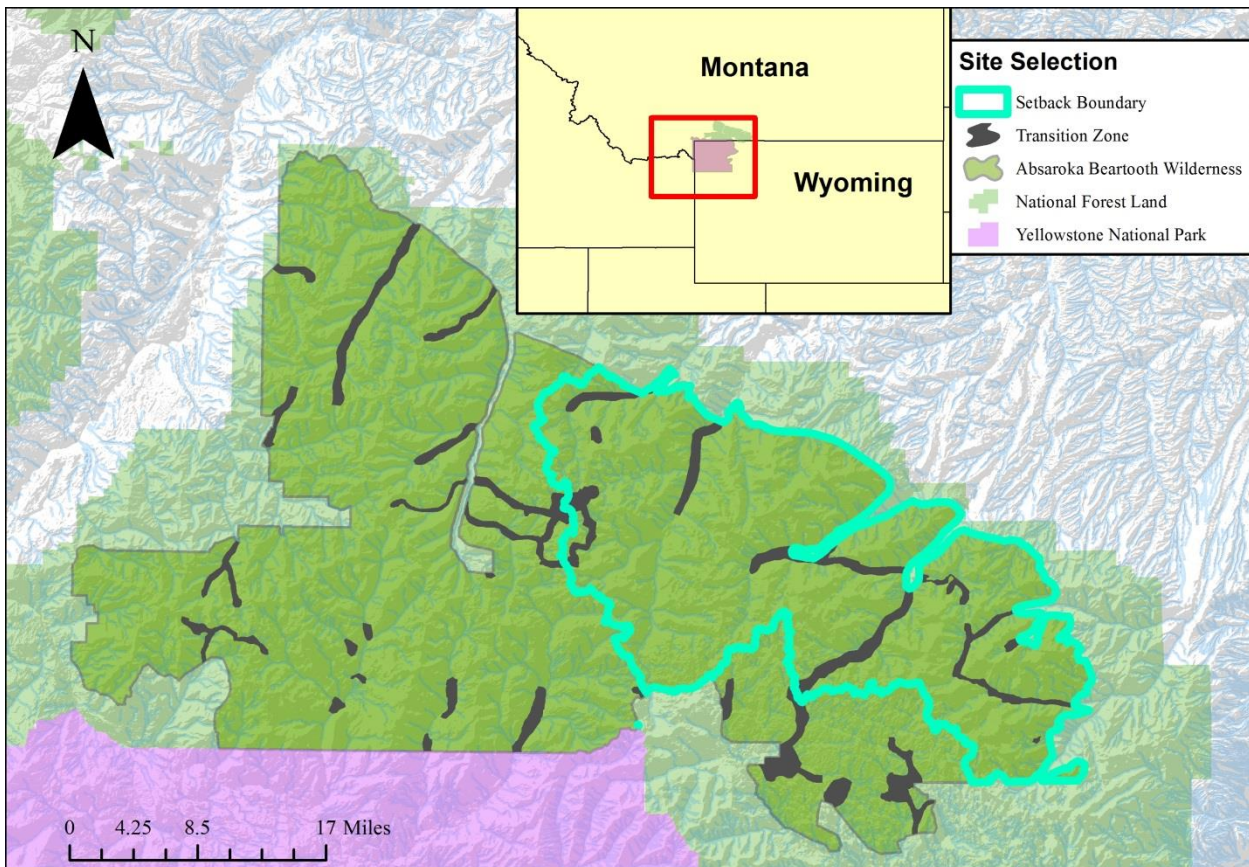


Figure 1. Location of the fecal contamination of Wilderness water resources study area in the Absaroka Beartooth Wilderness with an outline of the zone of setback requirements (in cyan).

The United States Forest Service on the Custer-Gallatin National Forest is concerned with the impact of backpacking on the natural character of the Absaroka Beartooth Wilderness (ABW). Over the past 20 years, the Custer-Gallatin has focused their Wilderness character monitoring efforts on the spread of invasive weeds and the physical impacts (e.g. vegetative cover lost, mineral soil exposed) from popular campsites degrading the “natural” measure of Wilderness character (Wilderness Institute

2013). Managers are concerned that high recreational use at alpine lakes and along popular trails is causing damage to natural resources such as native vegetation and water quality (Wood, personal communication, February 12, 2018).

Impacts to water quality are a major concern of the ABW managers due to the issues associated with congestion of campsites at popular water sources and subsequent water contamination from campers (Cilimburg 2000). To prevent impacts to water sources, managers in the ABW rely on education, restrictions, and enforcement (Wood, personal communication, February 12, 2018). Although the Custer-Gallatin has been proactive in their attempts to limit impacts to water resources, few assessments on water quality have been conducted within the ABW (Wood, personal communication, February 12, 2018). Whether or not current interventions such as setbacks are effective at deterring water contamination are, for the most part, unknown.

The ABW has a diverse management structure in that the Wilderness is divided into four jurisdictions: Clark's Fork, Gardiner, Yellowstone, and Beartooth ranger districts (USFS 2017). The Beartooth ranger district, located in Red Lodge, MT, has adopted specific setback guidelines with the goal of protecting water resources from recreational impacts (Wood, personal communication, February 12, 2018). On the Beartooth district, camping setbacks require visitors to camp 100 ft from streams and 200 ft from lakes to protect water resources from recreational impacts, including the fecal matter contamination of backcountry campers (USFS 2011). The other three ranger districts that comprise the ABW have no setbacks in place.

The uneven adoption of setbacks in the ABW is the product of the shifting management structures imposed by the consolidation of National Forests. The Absaroka Beartooth Wilderness was originally spread across two separate national forests with different sets of regulations: Custer National Forest and Gallatin National Forest (Nash 2017). Now that the two previously distinct entities are consolidated under one National Forest, Wilderness managers deciding whether to institute camping

setbacks from the old Custer National Forest for the whole Wilderness or eliminate the regulations altogether (Wood, personal communication, February 12, 2018). While it is assumed that camping setbacks protect Wilderness water resources, there are little empirical data to support these conclusions (Marion et al., 2018).

## **Fecal Water Contamination in the Absaroka Beartooth Wilderness**

Human waste occurrence is ubiquitous at campsites throughout the ABW due to improper waste disposal (Wood, personal communication, February 12, 2018). During monitoring efforts on the ABW and routine Wilderness Ranger patrols, employees and partners have reported rampant accumulation of waste at popular camping sites. Wilderness rangers are reported to spend a considerable time of their working day burying human waste away from sensitive areas such as water sources (Crootof, personal communication, February 12, 2018). The USFS is concerned about the amount of time employees are dealing with human waste and the subsequent risks to water resources, personnel, and visitors.

In 2015, managers on the Beartooth District of the ABW did an exploratory analysis on a few popular water sources in the Wilderness (Wood, personal communication, February 12, 2018). The water sources are located near Granite Peak (Wood, personal communication, February 12, 2018), the highest point in Montana and a popular destination for recreationists in the west (Bevan 1923). These sites were chosen in part due to the remote area which Granite Peak resides, complicating potential rescue scenarios for incidents with visitors (Wood, personal communication, February 12, 2018). The congestion at these areas can be severe, due to the popularity of summiting Montana's highest peak and the limited number of water sources in the area (Wood, personal communication, February 12, 2018).



The results of the analysis showed that both water sources had the presence of coliforms, indicating fecal contamination in the water sources (Wood, personal communication, February 12, 2018). Even though the site is in a high elevation alpine area, there are expanding herds of mountain goats in the area potentially adding to the total fecal contamination contribution (Flesch et al 2016). Despite counts of total coliform being high, managers are unable to definitively connect the fecal contamination to recreationists due to the presence of mountain goats and the lack of specificity of traditional fecal indicator bacteria (FIB) (Tambalo et al 2011).

Presence of total coliforms at these popular sites invoked interest among Wilderness managers to analyze other water sources in the ABW (Crootof 2019). Although Granite Peak is a very popular area in the ABW, other trails and destinations in the Wilderness also receive similarly high levels of visitor use. Other areas such as the Lake Fork-West Fork route and the Beaten Path trail are identified as areas with a disproportionate amount of recreational use and impact. Alpine lakes with fishing pressure are also a concern because of the sensitivity of alpine ecosystems and the aquatic resources they provide downstream (Farnlieter et al 2010).

Managers are interested in the occurrence of fecal contamination across all designated Wilderness to determine if management interventions are effective (Wood, personal communication, February 12, 2018). With limited time and resources, managers are looking to effectively focus their time and energy in an efficient manner to identify particularly problematic areas to concentrate their energy. Identifying areas with fecal contamination is important, but to effectively determine if management interventions have an effect on fecal contamination, managers must determine the source of fecal contamination through microbial source tracking.

## **Developing aquatic Wilderness Character monitoring component**



Wilderness character monitoring is essential for preserving Wilderness Character, because it allows managers to gather necessary data to assess current conditions and direct future management objectives. Although the data generated from this process is informative, managers must decide what to do with that information to inform future monitoring. A feedback loop should be in place for managers to use monitoring data to inform management decisions and to improve on the next round of monitoring. By adding an aquatic monitoring component to their Wilderness character monitoring, managers are using the data from years of monitoring and on the ground experience to identify specific impacts relevant to the ABW.

Managers in the ABW have already taken the first steps to develop an aquatic Wilderness character monitoring component by identifying human waste in water sources as a specific contaminant parameter to measure in the form of fecal indicator bacteria. The next step is to determine the occurrence of fecal indicator bacteria across Wilderness water resources to identify potentially hazardous water sources. Finally, to accurately determine the source of fecal contamination at popular water sources, managers could use microbial source tracking to properly determine if recreational use is degradation water quality. The combination of fecal indicator bacteria enumeration and a PCR-based assay provide an in depth assessment of fecal water contamination in the Absaroka Beartooth Wilderness.

## Questions and Hypotheses

To test if management interventions to protect water quality are having their desired effect on human waste contamination to water quality, upon total coliform/*E. coli* data collection and analysis I ask: How do setbacks influence the occurrence and quantity of fecal coliforms/*E. coli* in high use recreational lakes in the Absaroka Beartooth Wilderness? I hypothesize is that areas with setbacks

have less fecal contamination in adjacent water sources than non-setback areas as indicated by lower counts of total coliform/*E. coli* in sites in the setbacks group compared to the non-setback group.

Another question is: How does the physical condition of the land-water interface influence the occurrence of total coliforms/*E. coli* in high recreational use lakes in the ABW? I hypothesize that increasing visitor impact will result in increased fecal water contamination because total coliform and *E. coli* counts will be higher in water sources adjacent to more high use sites.

The final question is: What are the primary sources of fecal contamination in the Absaroka Beartooth Wilderness? My hypothesis is that we will find evidence of human fecal contamination in water sources adjacent to all high use sites.

## Next steps

In Chapter two, I apply the knowledge gathered and processed into chapter one to design and conduct an observational study in the Absaroka Beartooth Wilderness to help managers assess the potential connection between recreation and fecal water contamination.

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## Chapter 2

Manuscript to be submitted to the *Journal of Environmental Management* with the following co-authors: Daniel P. Pendergraph, Matthew J. Church, John Raineri, Lochlin Ermatinger, Adam Baumann, Alexander L. Metcalf, Thomas H. DeLuca, and Robert O. Hall.

### Introduction

Wilderness areas in the United States are unique in that there are restrictions on permanent facilities to manage visitor impacts and human waste. According to the Wilderness Act of 1964, designated Wilderness has restrictions on permanent structures, roads, and mechanized and motorized equipment among other criteria (Zahniser 1964). The aim of these criteria is to maintain Wilderness character to ensure that future generations may experience Wilderness as when it was first designated. Although the lack of infrastructure is designed to maintain Wilderness character, it challenges efforts to manage the impact of recreation on environmental resources, especially water quality (Cilimburg et al 2000).

Wilderness visitation has progressively increased since the 1960s (Cole 1996; USFS 2017), creating challenges for managers seeking to minimize impacts of recreational activities on sensitive habitats. Recreational impacts on protected areas are well studied in the field of recreation ecology (Cole et al. 1996; Monz et al 2010; Marion et al. 2016), including investigations of visitor impacts to cultural sites, vegetation, soil, wildlife, and water quality within protected Wilderness areas. (Cole and Landres 1996; DeLuca et al. 1998; Liddle 1997; Hammit et al. 2015). Such studies have promoted management efforts focused on mitigating impacts through educational outreach, Wilderness rangers, trail restrictions, and group size limits, among others (Marion et al. 2016; Hammit et al 2015)

A central concern among Wilderness managers is the impact of human recreation on water quality, primarily because visitors rely on these water sources for basic uses such as drinking, cleaning, and cooking. Among the impacts to water quality associated with visitation are increased sedimentation,

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introduction of anthropogenic products (*e.g.*, caffeine, micro-plastics), nutrient enrichment, and fecal contamination (Ursem et al. 2009; Madej et al. 1994, Clow et al. 2011, 2013). Fecal contamination and outbreaks of illnesses associated with bacteria (*e.g.*, infectious diarrhea, cholera) are well documented in congested sites of protected areas (McLaughlin et al. 2005; Meyer et al. 2016; Khan et al. 2018). Medical emergencies in remote parks and Wilderness are particularly troublesome due to the complex logistics of backcountry rescue.

Accumulation of human waste in aquatic systems is a growing problem globally and protected area managers must identify areas of fecal contamination in water resources to protect public health (Meybeck 2003; Apollo et al. 2017). Water resources adjacent to areas of high congestion sites are at the risk being contaminated by fecal bacteria such as total coliform/ *Escherichia coli* (*E. coli*) (Climburg et al. 2000, Clow et al. 2011). To properly mitigate fecal water contamination, managers must identify areas with occurrence of fecal bacteria and determine the source of contamination (Tamabalo et al. 2011; Paruch et al. 2015). Standardized methods such as fecal indicator bacteria (*e.g.*, total coliform/*E. coli*) and PCR-based assays (*e.g.*, Bernard and Field 2000; Layton et al. 2006) provide methods for detecting fecal contamination. PCR based assays have rapidly evolved over the last two decades from novel technologies to mainstream management tools (Tambalo et al. 2011; Paruch et al. 2015).

### *Fecal Indicator Bacteria*

Coliform bacteria mostly occur in the intestines of warm-blooded mammals, but can persist in the natural environment and are used globally as designated standard fecal indicator bacteria (FIB) in aquatic and terrestrial ecosystems (Farnleitner 2010; Pope et al. 2003; Paruch et al. 2015). There are three classes of coliform bacteria: total coliform, fecal coliform, and *E. coli*. While total coliforms can be introduced from a variety of different sources in the environment, the specific occurrence of *E. coli* more exactly indicates fecal contamination (APHA 2005). Testing for total coliform/*E.coli* is an efficient way to determine whether a water source is a public health hazard since testing for individual pathogens can be

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costly and time consuming (Derlet and Carson 2006). *E. coli* contamination in water resources poses a serious public health risk and although not all strains of *E. coli* are harmful to humans, many strains can cause illness (*e.g.*, infectious diarrhea) and certain strains of *E. coli* (*e.g.*, *E. coli* 0157:H7) have resulted in human fatalities (Chekabab et al. 2013). Detection of total coliform/*E. coli* provides an indication of fecal contamination (and associated pathogens) allowing for targeted management interventions to identify the source of contamination to improve water quality. Although water resources are used for some recreational activities (*e.g.*, fishing and swimming), water for drinking, cleaning, and cooking are the most ubiquitous uses in Wilderness areas. Under EPA standards for drinking water, if *E. coli* is detected through water sampling that particular water source is deemed a public health hazard (APHA 2005). Wilderness water resources have been analyzed in several studies and indicate that *E. coli* is most likely to occur in water sources adjacent to areas with high recreational use (Seimer 1968; Derlet and Carson 2004, 2006; Clow et al. 2011, 2013; Reed and Rasnake 2016).

### *Applying PCR-based methods for microbial source tracking in Wilderness waters*

*E. coli* is an effective indicator of fecal contamination and is widely used as a FIB in surface waters (Paruch & Mæhlum 2012), it is non-host specific (Tambalo et al. 2011). Increasingly, culture-independent analyses utilizing host specific genetic markers in microbial source tracking (MST) have been utilized to determine the source of the fecal contamination. Advancements in genetic tools such as the polymerase chain reaction (PCR) and environmental DNA (eDNA) over the last 15 years have made these technologies more accessible to environmental managers and are becoming broadly incorporated into monitoring protocols by agencies like the Environmental Protection Agency (EPA) (Shanks 2018).

Although *E. coli* is frequently utilized as an FIB, there are diverse strains of this organism that do not appear to be host specific, complicating its use for MST (Griffith et al. 2003; Meays et al. 2004). In contrast, a number of studies indicate that some members of the genus *Bacteroides* demonstrate host-specificity, and are increasingly useful for the MST of fecal contamination in surface waters (Bernhard

and Field 2000; Tambalo et al. 2011; Paruch et al. 2015; Kabiri et al. 2016). Enteric members of the genus *Bacteroides* can comprise upwards of 26–36% of host fecal microbiota, and are highly host-specific (Fiksdal et al. 1985; Kreader 1995; Bernhard and Field 2000). Moreover, enteric members of the *Bacteroides* are obligate anaerobes, making their replication in well-oxygenated environments unlikely. Thus, these organisms appear optimal candidates for MST of recent fecal contamination (Bernhard & Field 2000; Hold et al. 2002; Dick et al. 2005). Several *Bacteroides* host-specific markers have been developed and successfully applied in PCR assays worldwide to determine sources of fecal contamination, including humans, ruminants, cattle, pigs, and horses (Dick et al. 2005; Layton et al. 2006; Reischer et al. 2007; Shanks et al. 2008; Lamendella et al. 2009).

To date, *Bacteroides* makers have been successfully applied to agricultural, rural and urban water resources to determine sources of fecal contamination to these waters (Sauer et al. 2011; Tambalo et al. 2011, Paruch et al. 2015). The application of MST in a Wilderness setting provides an opportunity to investigate the biological contribution of human recreation to fecal contamination in water.

## Study Purpose and Objectives

This study had two primary objectives. First, to quantify the occurrence of total coliform/*E. coli* in water sources adjacent to popular camping areas in the Absaroka Beartooth Wilderness. Second, I will develop a PCR-based assay for the detection and quantification of human-associated fecal bacteria in Wilderness lakes and streams. To address these objectives, we used fecal indicator bacteria for occurrence of fecal contamination and applied *Bacteroides* host-specific primers for digital droplet PCR-based assays to evaluate potential sources of FIB in Wilderness waters.

## Methods

### *Study Area*

The Absaroka Beartooth Wilderness (ABW) is northeast of Yellowstone National Park in Montana and Wyoming, USA. This rugged, mountainous Wilderness contains extensive subalpine forests with a large contiguous alpine tundra component. The ABW receives high recreational use, mostly in the form of overnight backpacking. Most of the backpacking is concentrated around alpine lakes and at lakes near popular summits within the Wilderness area.

### *Site Selection*

I selected sites using geospatial campsite data as well as input from Wilderness managers. Campsites in the ABW are cataloged and monitored by U.S. Forest Service personnel and agency partners for the past 20 years to assess trends in visitor impacts via GPS and ArcGIS. Potential sampling sites were identified using ArcMap 10.5.1 by overlaying all campsite point locations within the ABW with a Wilderness wide opportunity class layer, representing three zones of recreational opportunity: 1) Pristine; 2) Primitive; 3) Transition zones. Pristine zones are classified as zones with negligible anthropogenic influence. Primitive zones are zones with measurable anthropogenic influence, but ecological processes (*e.g.*, water cycling) appear intact. Transition zones are defined as zones where human impacts on ecological conditions and processes were moderate to substantial. We used the geoprocessing clip tool to isolate large clusters of campsites within areas classified as transition zones to identify areas of congestion in high impact recreation zones.

Finally, we consulted with Wilderness managers to identify water sources adjacent to these areas that are potential candidates for fecal water contamination intervention strategies such as overnight camping permits or setbacks for camping near water sources (Figure 2).

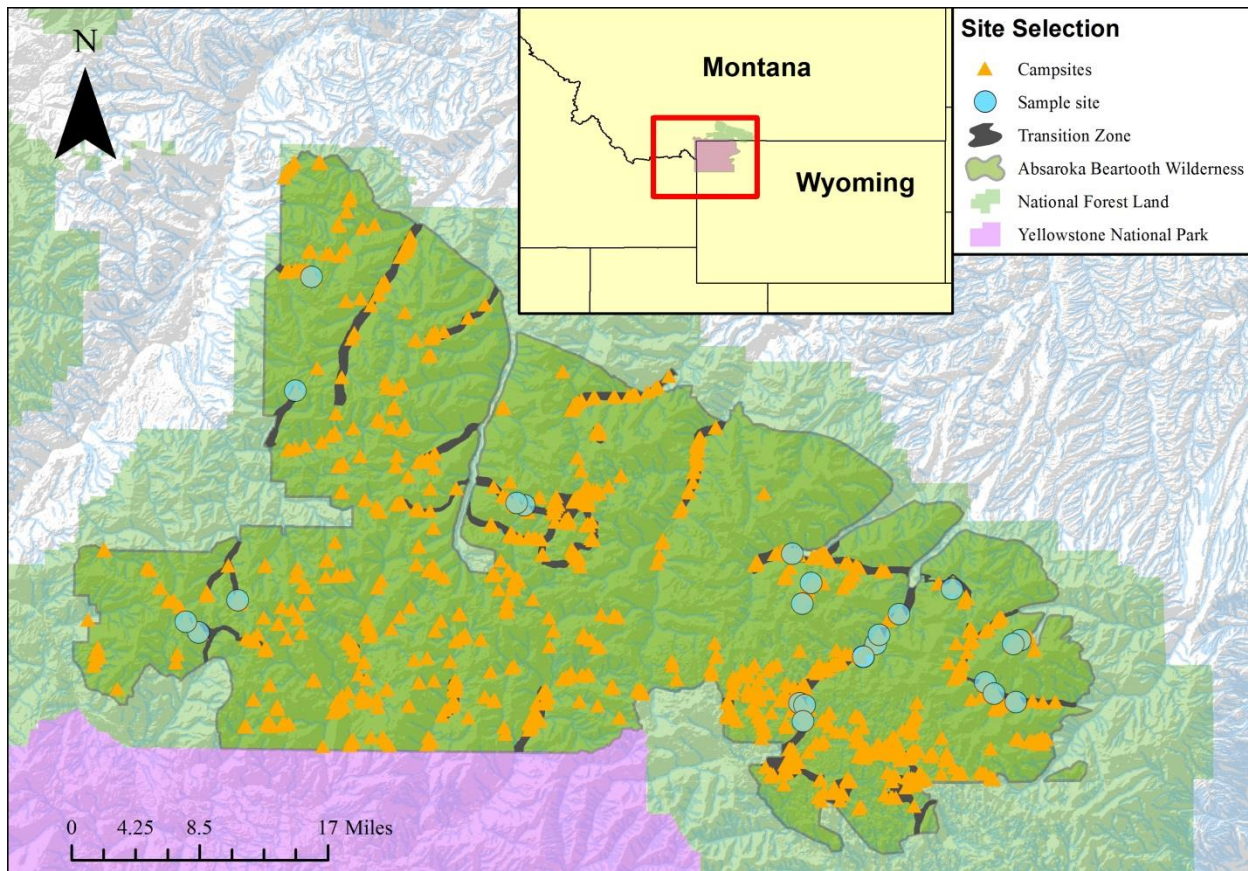


Figure 2 Study sites across the Absaroka Beartooth Wilderness.

### *Surface water sampling and sample preparation*

We collected surface water samples collected in triplicate using three autoclavable, 250 ml polypropylene (PP) bottles at each of the selected sampling sites during July and August of 2019. Samples were transported to the laboratory on ice and held at 4°C in a laboratory refrigerator until processing within 48 hours of sample collection. In the laboratory, from each of the three 250 ml sample bottles per lake, approximately 100 mL were vacuum filtered onto two 47 mm diameter, 0.45- $\mu$ m pore size mixed cellulosic ester filters (Millipore Sigma, Burlington, MA) to capture potential fecal bacteria. One of the filters was used for immediate culture-dependent FIB analysis, and the other filter was used for



subsequent MST/PCR analyses. Filters for subsequent extraction of DNA were stored in 15 ml sterile centrifuge tubes and frozen (-20°C) until processed in the laboratory.

### *Fecal Indicator Bacteria Enumeration*

Using pre-sterilized 0.45 µm gridded membrane filters (Millipore Sigma, Burlington, MA), triplicate samples filtered from each sampling site were placed in M-ColiBlue24 broth petri-dishes and incubated overnight at 35°C for 24 hours according to the manufacture's specifications (Millipore Sigma, Burlington, MA). Upon enumeration of total coliform and *E. coli*, colony forming units (CFUs) were quantified by counting and recording the number of CFUs in red (total coliform) and in blue (*E. coli*).

### *DNA extraction for PCR assay*

DNA was extracted from filters using a modified version of the MasterPure DNA Purification Kit (Lucigen Corporation, Middleton, WI). Briefly, filters were transferred from 15 mL centrifuge tubes to 2 ml microcentrifuge tubes containing 600 µL of Tissue/Cell Lysis Solution and 100 µl of 0.1 mm and 100 µl of 0.5 mm glass beads. Tubes containing filters were frozen at -80°C, then thawed, and placed into a mechanical bead beater for 2 minutes to facilitate physical disruption of cells. Microcentrifuge tubes were removed from the bead beater, pulse centrifuged, followed by addition of Proteinase-K (50 µg/µl final concentration). Tubes were incubated at 65°C for 15 minutes, vortexed every 5 minutes, and then placed on ice for 3-5 minutes. DNA was subsequently extracted from samples following the MasterPure DNA Purification kit protocols. The resulting DNA was resuspended in 100 µl of either Tris-EDTA buffer or DNase/RNase free water. DNA extracts were stored at -80°C until PCR analyses.

*Digital droplet PCR (ddPCR) assay for detection of universal and human specific Bacteroides markers in Wilderness water sources*

Oligonucleotide sequences of primers used for ddPCR assays are listed in Table 1. For the current study, we used conserved primers designed to target 16S rRNA genes of members of the genus *Bacteroides* derived from human, cattle, and equine feces (AllBac), and more specific primers designed to target 16S rRNA genes of only human-associated *Bacteroides* spp. (Bernhard & Field 2000; Hold et al. 2002; Dick et al. 2005; Paruch et al 2015). Oligonucleotide sequences of primers used for ddPCR assays are listed in Table 1. All ddPCR assays were performed in triplicate using a QX200 Droplet Digital PCR System (Bio-Rad Laboratories, Hercules, CA, USA). Each 20 µl reaction mixture contained 10 µl iQTM SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 7.2 µL of nuclease-free water, 2 µl of a 10-fold dilution of the template DNA, and 0.18 µmol/L (final concentration) of each primer. Triplicate no template controls (no added DNA) were included with each thermal cycling run.

16S rRNA gene target	Primer name	Sequence (5'-3')	References
AllBac ( <i>Bacteroides</i> )	AllBac 296f AllBac 412f	GAGAGGAAGGTCCCCAC CGCTACTTGGCTGGTTCAG	Layton et al. (2006)
BacH (humans)	BacH_f BacH_r	CTTGCCAGCCTTCTGAAAG CCCCATCGTCTACCGAAAATAC	Reischer et al. (2007))

**Table 1. PCR primer sequences utilized for microbial source tracking analyses of water samples collected from the Absaroka Beartooth Wilderness**

Reactions were amplified under the following thermal cycling conditions: 95°C for 6 minutes, followed by 40 cycles of 95°C for 30 s, 61°C for 30 s and 72°C for 45 s. After amplification, a melt curve profile was run to verify the specificity of the resulting PCR amplicons. Gene abundances (copies per ml of sample water) were quantified by fitting a Poisson distribution to the resulting fluorescence



measurements from each sample (QuantaSoft™, Bio-Rad Laboratories). No template controls (no DNA) and the sample

DNA were assessed in triplicate. A lower detection limit, defined as a two-fold increase in sample fluorescence relative to the blank, was 10 copies per reaction for both PCR assays.

## Results

### *Total Coliform and E. coli occurrence in Wilderness water sources*

Coliforms occurred in 21 of 21 (100%) lake outlets sampled, while *E. coli* was found in about half 11 of 21 (52%) of the sampled sites. Mean total coliform and *E. coli* CFUs, based on triplicate samples enumerated from each lake, ranged from 27 to >200 CFUS per 100 ml of lake water and from below detection to 23 CFUS per 100 ml of lake water, respectively. Overall, all 63 samples (100%) contained total coliforms whereas 20/ 63 (31.7%) of the samples also contained *E.coli*. Total coliform levels of >100 CFUS per 100 ml of lake water were detected in 42/63 (66.6%) sites of the sample (See Table 2).

Lake outlet	Total coliform (CFUS/ 100 ml)	<i>E. coli</i> (CFUS/100 ml)	Lake outlet	Total coliform (CFUS/ 100 ml)	<i>E.coli</i> (CFUS/100 ml)
Horseshoe	>200	1	Rainbow	82	0
Ouzel	62	0	Elk	152	2
Elbow	93	0	Thompson	129	0
Lake At Falls	142	1	Pine Creek	27	0
Rimrock	124	1	Timberline	149	2
Gertrude	174	1	September Morn	162	0
Sylvan	180	4	Bald Knob	89	0
Fish	>200	0	Knox	73	1
Mystic	85	0	Keyser Brown	>200	5
Diamond	144	0	Russell	127	1
Lost	192	23			

**Table 2 – Occurrence of Total coliform and *E. coli* in lake outlets of the Absaroka Beartooth Wilderness. Total coliform and *E. coli* reported in colony forming units (CFUS) per 100 ml of lake water.**

### *Application of universal and host specific Bacteroides MST markers in Wilderness water sources*

Two ddPCR assays were utilized using primers targeting *Bacteroides* species frequently found in human, cattle, and equine feces (AllBac) and human-specific *Bacteroides* 16S rRNA gene markers (BacH), was applied to sampling sites where *E. coli* was detected in the FIB occurrence analysis. Additionally, four lakes where total coliform was detected without confirmed *E. coli* presence were analyzed by ddPCR (Table 3; September Morn, Snowbank streams 1 & 2, and Diamond Lake).

Gene abundances were quantified when all three of the triplicate PCR reactions amplified above the lower limit of detection. We also qualitatively scored the positive presence of target genes in those samples where one or more of the triplicate ddPCR reactions amplified above the lower limit of detection.

In 9 of 15 (60%) sampled sites, we were able to quantify the number of AllBac gene targets; however, presence of AllBac gene targets were observed in all (100%) of the sites sampled (Table 3). For those sites where gene abundances were quantifiable, AllBac gene copies ranged from 144 to 1404 per ml of sampled water.

In contrast, only Elk Lake had quantifiable gene abundances for the human-specific BacH ddPCR targets (Table 4). At this lake, human-associated gene copies were 33 genes/ ml of water. However, in 7 of the 15 (47%) sampling sites BacH gene targets were present but below the lower limit of quantifiable detection (Table 3).

Site	Quantifiable genes of AllBac (copies per 100 ml water)	Presence of Allbac	Quantifiable genes of BacHu (copies per 100 ml water)	Presence of BacHu
Diamond Lake	n/a	Yes	n/a	No
Elk Lake	325	Yes	33	Yes
Horseshoe Lake 1	158	Yes	n/a	No
Keyser Brown Lake	962	Yes	n/a	No
Knox Lake	n/a	Yes	n/a	No
Lake at Falls	289	Yes	n/a	Yes
Lake Gertrude	199	Yes	n/a	No
Lost Lake	921	Yes	n/a	Yes
Rainbow Lake	n/a	Yes	n/a	Yes
Rimrock Lake	n/a	Yes	n/a	Yes
Russell Lake	144	Yes	n/a	No
September Morn Lake	152	Yes	n/a	No
Snowbank 1	n/a	Yes	n/a	Yes
Snowbank 2	1309	Yes	n/a	No
Timberline Lake	n/a	Yes	n/a	Yes

**Table 3– Detectable occurrences of the *Bacteriodes* genetic makers AllBac and BacH across 15 sites in the Absoroka Beartooth Wilderness in south central Montana. For Presence of target 16s rRNA genes, “Yes” indicate samples that had a detectable signal but were not quantifiable, i.e., at least one of three replicate reactions resulted in detectable amplification; “n/a” indicates where there was no detectable amplification in any of the triplicate reactions/**

## Discussion

### *Occurrence of fecal contamination at popular camping locations*

We observed occurrence of total coliform in all of the lake outlets sampled. Such results likely reflect the open nature of these habitats by receiving waters with the surrounding landscape. Coliforms derive from diverse environmental sources, including numerous non-fecal sources (Procopio et al. 2017). Hence the detection of coliforms in these water samples does not necessarily indicate fecal contamination but rather untreated water that is exposed to the open environment.

The positive occurrence of *E. coli* in over half the lake provides a more definitive assessment of fecal contamination since *E. coli* lives in the gastrointestinal tract of animals. The detection of *E. coli* in our sampling sites is concerning because it is an abundant anaerobic gastrointestinal flora as well as the cause of a significant fraction of human bacterial diseases (Siitonen 1994; Leclerc et al. 2001; Gordon et al. 2002). The presence of *E. coli* in several of the Wilderness sites sampled as part of this study suggests recent fecal contamination. Studies have correlated both total coliform and *E. coli* counts to times of peak recreational use of Wilderness water sources (Derlet and Carson 2003,2006;Forester and Scott 2016).

Results from these studies suggest occurrence of *E. coli* may be related to human activity; however, results from our study do not support this conclusion because *E. coli* is non-host specific. The occurrence of *E. coli* in any water source indicates likely fecal contamination, providing motivation for further investigation into possible sources. Regardless, fecal bacteria contaminated some Wilderness lake outlets, serving as potential health hazards for Wilderness visitors and obviates the need for water filtration or purification prior to consumption.

### *Application of a ddPCR-based assay for the detection and quantification of Bacteroides in Wilderness water sources*

Due to remote sampling locations and the logistics of transporting samples back to the laboratory for processing, we opted to filter 100 ml of water per sampling site for subsequent PCR analyses. Previous studies utilizing these same PCR primers generally filtered a larger volume of water (Tamabalo et al., 2011; Paruch et al 2015). The resulting limit of detection for our ddPCR assays was 10 gene copies per ml of sample water, compared to 25 genes per ml of water from previous studies that have utilized quantitative PCR for detection of these gene targets (Paruch et al. 2015). The loss of sensitivity in our case was likely due lower volumes of water filtered compared to these previous studies. Nonetheless, generally low abundances of the target microorganisms occurred at our lakes. Future studies in relatively

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pristine Wilderness habitats would benefit from increased filter volumes to decrease the lower the limit of gene quantification by PCR-based assays.

Our use of the AllBac ddPCR assay demonstrated that most (60%) of lake outlets sampled as part of our study have feces-associated members of the genus *Bacteroides* ranging from 152-1309 copies per 100 ml of water. Together with our *E. coli* occurrence data, such results highlight that even the relatively remote alpine lakes sampled as part of this study can contain pathogenic microorganisms. Notably, only one of the lake outlets (Elk Lake) sampled in our study resulted in quantifiable gene abundances for human-feces associated *Bacteroides*. However, the presence of human-feces associated *Bacteroides* spp. were observed at several other of the sampling sites. Elk Lake was one of the sites closest to the main trailhead into this Wilderness area, and this sampling site is downstream of several popular lakes that tested positive using the ddPCR assay.

In our assessment of Wilderness water sources, we sampled mostly alpine lake outlets; however, we also sampled two snowmelt streams near Granite Peak, the highest point in Montana and a popular summit within the Wilderness. The scarce amount of water around Granite Peak may create high congestion basecamp areas and in turn may put disproportionate pressure on water resources adjacent water sources. Both snowmelt streams tested positive for the presence of feces-associated members of the genus *Bacteroides* (based on the AllBac PCR assay). Moreover, human-feces associated *Bacteroides* spp. occurred in one of the snowmelt streams (Snowbank 1, Table 3). The detection of human-derived fecal bacteria in streams suggests a need for the investigation of the presence of such organisms in streams, seeps, and springs as well as lake outlets to identify other water sources experiencing human fecal contamination. High counts of Allbac but absence of BacH in Snowbank 2 reflects influence of animals on fecal contamination in water resources, further highlighting the vulnerability of these high elevation streams to pathogenic microorganisms. Results of our FIB analysis and ddPCR assay on both streams and

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lakes highlights potential hazards associated with drinking untreated water from both lotic and lentic water sources.

## Conclusion

Although Wilderness water sources are frequently characterized as high quality due to the lack of permanent anthropogenic presence within the designated boundaries, short-lived human recreation activities can have significant impacts to water quality. Widespread occurrence of coliforms and *E. coli* in the Wilderness highlight the importance of water purification in both running and standing water to avoid exposure to harmful bacteria.

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## Chapter 3

### Introduction

To investigate the influence of recreation and managerial interventions on fecal water contamination in the Absaroka Beartooth Wilderness I conducted a number of statistical hypothesis tests. This provides us a rigorous way to test the data and identify potential improvements with the entire data process and design. Next, I will compare the total coliform/*E. coli* occurrence results of the Absaroka Beartooth study to the results of other fecal water contamination studies conducted in Wilderness and as well as non – capital ‘W’ Wilderness backcountry areas to identify communities and differences in results and study design. I can then identify trends across these studies and tease out methodological discrepancies between studies that may make a synthesis of the issue of fecal water contamination across Wilderness areas more difficult. Then I will review our use of digital droplet polymerase chain reaction (ddPCR) in this current application and potential future uses of this technology in managing water quality. ddPCR is a state-of-the-art tool that will enable us to answer powerful questions more definitively than traditional fecal indicator bacteria methods. Finally, I will reflect on the process of PCR assay as well as the data to improve these methods. Ending with a reflection of these results and possible management implications, I will recommend some potential avenues for future research design and next steps in assessing fecal water contamination in Wilderness and beyond.

### Initial Questions: Statistical Hypothesis Testing

*How does the physical condition of the land-water interface influence the occurrence of total coliforms/*E. coli* in high recreational use lakes in the ABW?*

To test our hypothesis that recreational activities increase the occurrence of total coliform/*E. coli*, we conducted the non-parametric Kendall correlation test to determine the effect of barren core (i.e. the area of impacted ground due to visitor use) on counts of total coliform and *E. coli*. Barren core (sq. ft.) is a metric used by the United States Forest Service to determine the amount of vegetation worn away at a site

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due to recreational activities by visitors. To normalize and scale for each lake we divide barren core by the surface area of each lake to derive the “Barren core ratio”. In the Absaroka Beartooth Wilderness(ABW), managers have been monitoring barren core for several years and barren core data is available for each site in our data set to accompany our fecal indicator bacteria occurrence data. Our Kendall correlation test looking at the effect of barren core on total coliforms revealed a tau of 0.186 and a 2 sided p-value of .26906, providing no evidence of barren core increasing total coliform counts in adjacent water sources (see Figure 1).

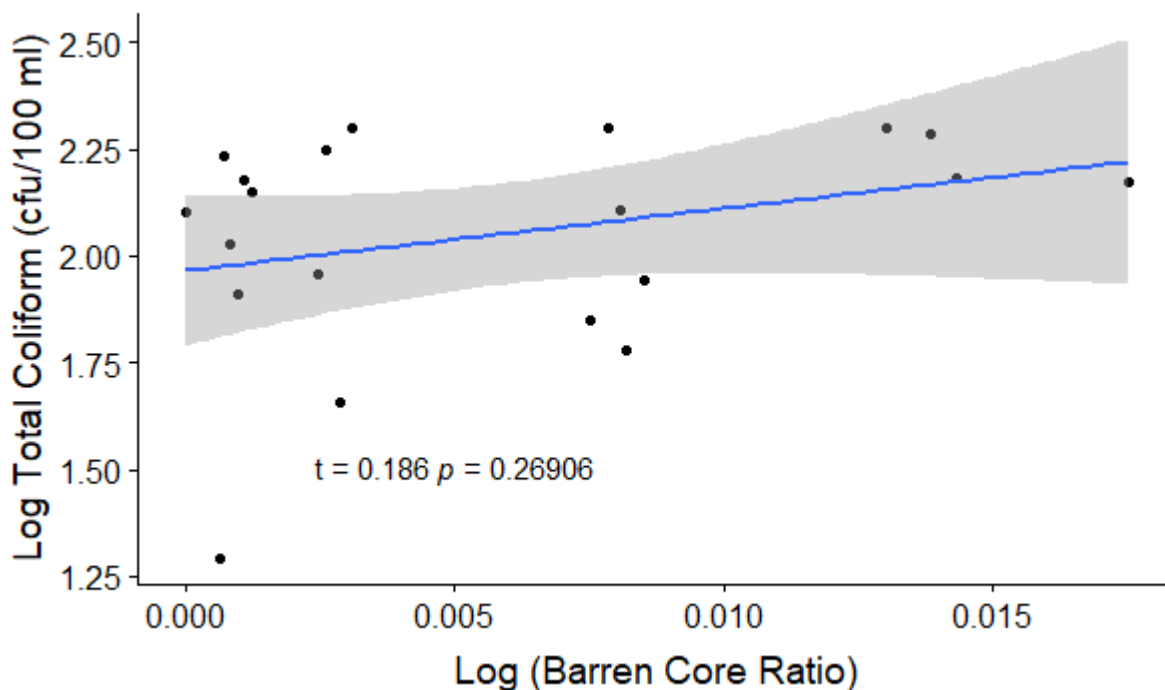


Figure 1- The relationship of the log Barren Core ratio on log total coliform counts (cfu/100 mL of water)

When we conducted the same test on the effect of barren core on *E. coli*, we found a similar result of a tau of 0.154 and a p-value of .039228 (figure 2), also suggesting no evidence of increasing barren core on *E. coli* counts in adjacent water sources. We used the Kendall correlation test due to the skewed distributions of both total coliform and *E. coli*. These results are skeptical at best due to low sample sizes.

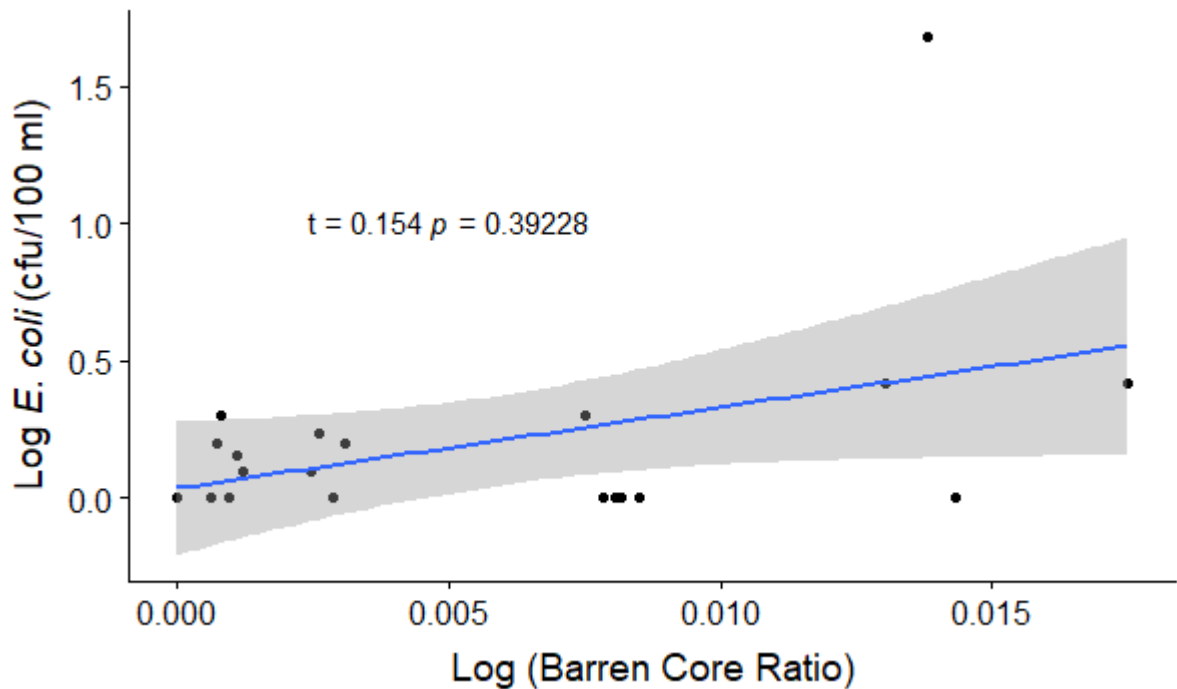


Figure 2- The relationship of the log Barren Core ratio on log *E. coli* counts (cfu/100 mL of water)

*How do setbacks influence the occurrence and quantity of fecal coliforms/E.coli in high use recreational lakes of the Absaroka Beartooth Wilderness?*

To determine if sites with setbacks are experiencing less fecal contamination than sites without setbacks, we conducted a Wilcoxon sign ranked test to compare these two populations to determine if there is an effect of setbacks on sites experience fecal contamination. One intervention Wilderness managers often employ to protect water resources is the use of camping setbacks. Camping setbacks vary across protected areas and can have conflicting restrictions in the same protected area.

In the Absaroka Beartooth Wilderness(ABW), camping setbacks are only present in one of the four jurisdictions that manage the Wilderness. In the Beartooth ranger district, setback restrictions require users to camp 100ft away from streams and 200ft away from lakes in order to protect water quality and limit damage to riparian areas (USFS 2019). The effectiveness of setbacks has long been questioned by Absaroka Beartooth Wilderness managers (Wood, personal communication, February 12, 2018) since there are data suggesting that, Wilderness wide, people are not following setback restrictions.

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Our test revealed that we did not observe results supporting our alternative hypothesis that the distribution of counts of total coliform/*E. coli* in lakes with setbacks (n=10) would be shifted to the left of that of lakes without setbacks (n=10). In fact, the distribution of *E. coli* counts in lakes with setbacks was shifted to the right of the distributions of *E. coli* counts in lakes without setbacks (p-value= 0.9953) (see figure 3).

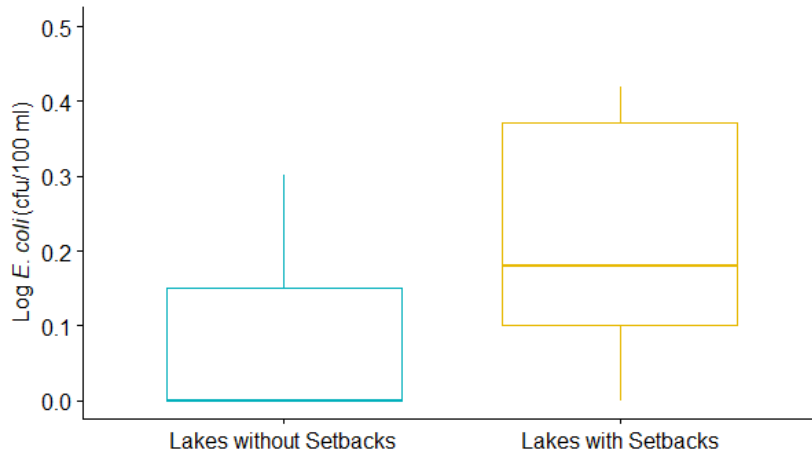


Figure 3: Log *E. coli* counts in Lakes without setbacks and Lakes with setbacks in the Absaroka Beartooth Wilderness in south central Montana.

In addition, the distribution of total coliform counts in lakes with setbacks was shifted to the right of the distributions of total coliform counts in lakes without setbacks (p-value=0.9978) (see figure 4). These results suggest that setbacks are not having the intended effect of mitigating fecal contamination on water sources.

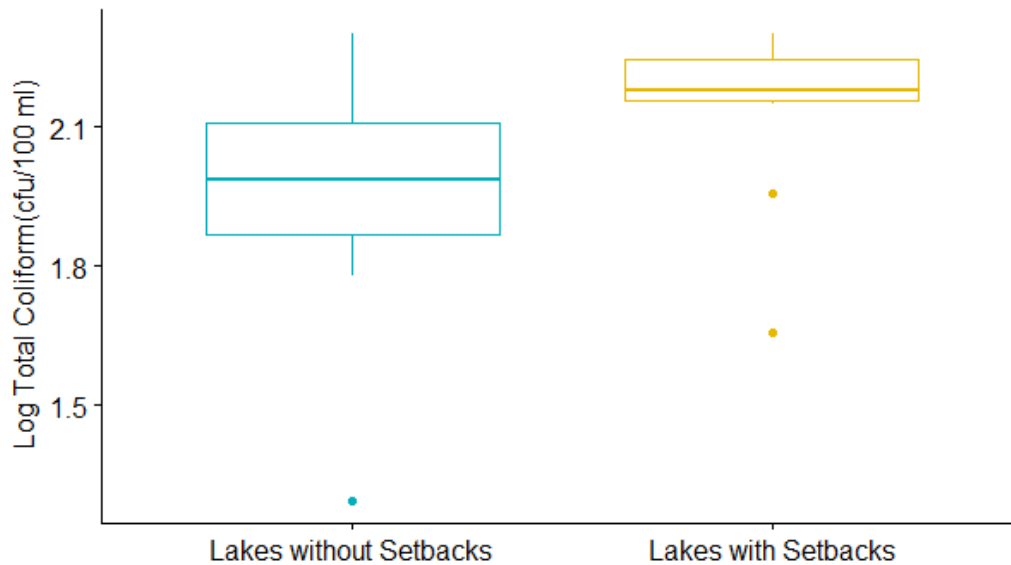


Figure 4: Log coliform counts in Lakes without setbacks and Lakes with setbacks in the Absaroka Beartooth Wilderness in south central Montana.

## Occurrence of total coliform/*E. coli* in the Absaroka Beartooth and beyond

### Occurrence of total coliform and *E. coli* counts in lake outlets of the Absaroka Beartooth

Wilderness somewhat reflect the levels found in other aquatic fecal indicator bacterium (FIB) analyses in the popular backcountry areas of the Sierra Nevada, Smokey Mountains, Grand Teton National Park and Cairngorms Park in the UK (Derlet and Carson 2006; Derlet et al., 2008; Forrester and Scott 2016).

Although some of other studies had high occurrence of total coliform in their samples (e.g., Cairngorms Park with coliforms in 85% of their sample), none had the absolute occurrence of coliform in 100% of their samples as in our study. *E. coli* occurrence in our water samples tended to be less than that of two similar studies with *E. coli* occurring in 46% our total sample compared to 75% at Cairngorms Park and 60% in the Smokey Mountains.

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Lake outlet	Total coliform (CFUS/ 100 ml)	<i>E. coli</i> (CFUS/100 ml)	Lake outlet	Total coliform (CFUS/ 100 ml)	<i>E.coli</i> (CFUS/100 ml)
Horseshoe	>200	1	Rainbow	82	0
Ouzel	62	0	Elk	152	2
Elbow	93	0	Thompson	129	0
Lake At Falls	142	1	Pine Creek	27	0
Rimrock	124	1	Timberline	149	2
Gertrude	174	1	September Morn	162	0
Sylvan	180	4	Bald Knob	89	0
Fish	>200	0	Knox	73	1
Mystic	85	0	Keyser Brown	>200	5
Diamond	144	0	Russell	127	1
Lost	192	23			

**Figure 5 Total Coliform and *E. coli* occurrence across the Absaroka Beartooth Wilderness**

The higher total occurrence of coliform in this analysis may reflect the fact we sampled exclusively from high use lake outlets in our FIB analysis; while other studies focused on sampling a spectrum of sites from high to low use as well as sampling both lakes and streams. For example, Derlet et al. (2008) sampled Wilderness water sources in the Sierra Nevada and found that sites with lower recreational use were less likely to have occurrence of coliforms in repeated water sampling. A possible explanation for this could be that in other studies such as in the Sierra Nevada, water samples were not analyzed within the range of recommended holding times and temperatures for coliform bacteria (Derlet et al., 2008) whereas with this study all samples accommodated recommendations by Pope et al. (2003).

The reason for the lower occurrence of *E. coli* in the Absaroka Beartooth Wilderness could be the two different methods for measuring and analyzing bacteria in water. In the Cairngorms Park and the Smokey Mountains, the metric the researchers used to quantify total coliform and *E. coli* were using was the most probable number (MPN) while in our study we used colony forming units (CFUs). The difference between the two methods is that CFU are the actual count from the surface of a plate, while MPN is a statistical probability of the number of organisms in the sample (American Public Health 2012). While the MPN and CFUs are reported to be equal in equivalent concentrations of *E. coli* in water samples (e.g. 1 MPN per 100 ml = 1 CFUs per 100ml) (Idexx 2017), this may not be the case. Water samples containing identical *E. coli* concentrations in surface water samples are quantified and compared



using both methods, seasonal (fall) MPNs estimates of *E. coli* densities may exceed counts of CFUs by an order of magnitude (Cho et al., 2010).

### **Discrepancies in fecal indicator bacteria methodology across studies**

To accurately characterize the issue of fecal contamination across Wilderness areas, the methods of measuring analyzing total coliform and *E. coli* need to be standardized. The inconsistencies in how total coliform/*E. coli* are measured by researchers makes a synthesis of the issue of fecal contamination in Wilderness water difficult. Not only are researchers measuring and reporting total coliform/*E. coli* differently but also are sampling very infrequently due to the logistics of accommodating holding times and the remote sampling locations of these sites. Accommodated holding times in these wilderness water studies ranged from under 24 hours to over a week (Derlet et al., 2008; Clow et al., 2013; Forrester and Scott 2016). Pope et al., 2003 revealed that after 48 hours *E. coli* densities can decrease significantly when samples were held at 4°C.

Derlet et al (2008) went so far as to wait up to a week holding samples at 35°C before analyzing for total coliform and *E. coli*. Their justification for waiting this long was that they conducted a control study and found that colony survival was not affected after one week of holding at 35°C and cited this study as unpublished data in their paper. This is in direct opposition to Pope et al. 2003, who suggested that samples over multiple sites had lower *E. coli* densities after 24 h at temperatures ranging from 4°C to 35°C. Moreover, in Derlet et al., (2008), samples were filtered and analyzed at a volume 1 ml of water then CFU values were extrapolated to per 100 ml water by multiplying the values at 1 ml by 100. Although this method is utilized in various situations in water research, it still is an extrapolation and should be treated as such and the associated process error should be taken into account more seriously along with the other issues of process and observation error regarding the Derlet et al., (2008) study.

## Shortfalls of using fecal indicator bacteria enumeration for source tracking

Fecal indicator bacteria indicate recent fecal contamination in water and specify which potential pathogenic bacteria that may be present in the water (Pope et al., 2003; Paruch et al., 2015). The problem arises, however, when trying to source track with bacteria with low host specificity and that have facultative physiologies (Paruch et al., 2015). In addition, the holding condition requirements add additional constraints to the sampling process especially when sampling in remote locations such as remote Wilderness lakes.

Many managers attempt to source track using fecal indicator bacteria enumeration, the standard FIB method of growing fecal bacteria from samples has his disadvantages. First the low-host specificity in standard fecal bacteria indicators prevents the researcher from definitively determining the organism responsible for the contamination. For example, *E. coli* which is used for fecal indicator bacteria comes from a number of different sources including domestic livestock, humans and wildlife. Other fecal bacteria indicators with higher host specificity have been suggested for use, for example bacteria within the genus *Enterococcus*, but even *Enterococci* have been proven to have multiple different species derived from human gut microflora making it difficult to select for one fecal bacteria indicator (Layton et al 2010). Recent studies have also suggested that the *Enterococci* are not as host specific as originally thought and may be present in other select animal hosts (Layton et al., 2009; Whitman et al., 2007). Although these are the traditional and still standardized methods for determining fecal contamination by various land management agencies, other methods such as microbial source tracking using genetic makers including PCR are gaining traction (Fuijoka et al., 2015).

## **Change of focus: What is the source of fecal contamination in the high use recreational lakes of the ABW?**

After detecting high occurrence of fecal indicator bacteria, we convinced our funding partners to invest in host specific genetic methods since total coliform/*E. coli* are non-host specific therefore not definitive source trackers. The benefit of source tracking with host specific methods such as polymerase chain reaction (PCR) is that it eliminates our needs to infer contamination from statistical hypothesis testing using something like barren core as a proxy for visitor use. By using PCR we can definitively link humans to recreation, eliminating potential error from trying to infer causation from correlation.

PCR was developed in the mid-1980s and since has evolved from a novel technology to an applied tool for host-specific source tracking in aquatic research (Saiki et al., 1985; Tambalo et al. 2011; Paruch et al., 2015; Pendergraph et al., (in review)). PCR allows researchers to synthesize large amounts of DNA by targeting the 16s rRNA genes used in reconstructing phylogenetic information. Forward and rear primers are designed to bind to conserved and hypervariable sites on the 16s rRNA for synthesis of targeted DNA. If the targeted section of the 16s rRNA gene is present, the DNA will be synthesized and amplified to produce millions of copies of that particular DNA. PCR has been used in numerous applications from disease identification and monitoring, to cloning biological organisms to microbial source tracking (Logan et al., 2019).

Since its inception, PCR has provided definitive analyses for the source tracking of fecal contamination (Fuijoka et al., 2015). Increasingly, PCR assays are being developed for various tracking applications in different environments across the globe (Tambalo et al., 2012; Paruch et al., 2015). PCR based assays using targeting the 16s rRNA primers of the fecal bacteria *Bacteroides* have been applied to number of different ecological systems including urban, agricultural and now Wilderness settings. *Bacteroides* is an anaerobic fecal bacterium that resides in the intestines of many animals, making it an effective indicator of recent fecal contamination (Tambalo et al., 2012). By directing target the 16s

rRNA primers of *Bacteroides*, researchers are able to definitively identify *Bacteroides* by species via its phylogenetic attributes (Paruch et al., 2015).

The final objective of this study was to source track using a PCR-based assay using universal and host specific *Bacteroides* makers to compare the amounts of both these *Bacteroides* ssp. genes present in our sample. For our general non-host specific *Bacteroides* maker, Allbac, we were able to detect non host specific fecal contamination in all of the sites in our assay and quantify the gene copies for 9 of the 15 sites in our essay. The number of copies ranged from 144 copies/100 ml of water at Russell Lake up to 1309 copies/100 ml of water in one of two stream sites in our assay, Snowbank 2(See figure 6). This variability in copies/100 ml between the Russel Lake and Snowbank 2 may reflect the influence of dilution on densities of gene copies since Russell Lake is a relatively large body of water and Snowbank 2 is a small snowmelt stream/seep.

Our human specific *Bacteroides* marker, BacH, produced viable reactions in 7 of the 15 sites in our assay therefor provides a direct link between recreation and fecal water contamination in the Absaroka Beartooth. These sites were Timberline Lake, Snowbank 1, Rimrock Lake, Rainbow Lake, Lost Lake, Lake at the Falls and Elk Lake. We were only able to quantify gene copies for one lake, Elk Lake, with 33 copies per 100 ml of water. Elk Lake was the closest lake out of all of the sites to its respective trailhead and one of the lowest lakes on the Beaten Path a popular hiking trail, which may explain our ability to quantify gene copies at this site and not others. Rimrock, Rainbow, and Lake at the Falls are all located above Elk Lake in the same drainage, East Rosebud Creek, which suggests Elk Lake may be a zone of accumulation for human fecal matter since we were able to quantify human contamination at this site and only able to determine presence absence at other sites. Timberline Lake and Lost Lake are both high use and standalone lakes (i.e., lakes with no upstream influence from other lakes) which may explain our ability to detect BacH as well as our inability to quantify genes at these sites. The detection of BacH

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along the popular Beaten Path as well as in popular standalone areas highlights the link of human recreation to fecal water contamination at in Wilderness water resources.

Site	Quantifiable genes of AllBac (/100 ml water)	Presence of Allbac	Quantifiable genes of BacHu (/100 ml water)	Presence of BacHu
Diamond Lake	n/a	Yes	n/a	No
Elk Lake	325	Yes	33	Yes
Horseshoe Lake 1	158	Yes	n/a	No
Keyser Brown Lake	962	Yes	n/a	No
Knox Lake	n/a	Yes	n/a	No
Lake at Falls	289	Yes	n/a	Yes
Lake Gertrude	199	Yes	n/a	No
Lost Lake	921	Yes	n/a	Yes
Rainbow Lake	n/a	Yes	n/a	Yes
Rimrock Lake	n/a	Yes	n/a	Yes
Russell Lake	144	Yes	n/a	No
September Morn Lake	152	Yes	n/a	No
Snowbank 1	n/a	Yes	n/a	Yes
Snowbank 2	1309	Yes	n/a	No
Timberline Lake	n/a	Yes	n/a	Yes

**Figure 6- Detectable occurrences of the *Bacteriodes* genetic makers AllBac and BacH across 15 sites in the Absaroka Beartooth Wilderness. For Presence of target 16s rRNA genes, “Yes” indicate samples that had a detectable signal but were not quantifiable, i.e., at least one of three replicate reactions resulted in detectable amplification; “na” indicates where there was no detectable amplification in any of the triplicate reactions**

Snowbank 1, located on the Froze-to-death Plateau (figure 7) was one of our two snowmelt streams in the study. The Froze-to-death Plateau provides few base camp options for summiting Granite Peak, Montana’s highest mountain, since water sources become scarce as climbers near the summit. The detection of BacH in Snowbank 1 is alarming since it is only one of a few water sources available to climbers in this extensive area. Visitors in other areas may be able to avoid lake outlets by pulling water from source creeks but water sources like Snowbank 1 on the Froze-to-death Plateau are the top of the drainage and sourcing water above this source is not possible. High counts of Allbac but absence of BacH in Snowbank 2 reflects a major influence of wildlife on fecal contamination in water resources, further

highlighting the vulnerability of these high elevation streams to pathogenic microorganisms and obviating the need for water purification at these sites.

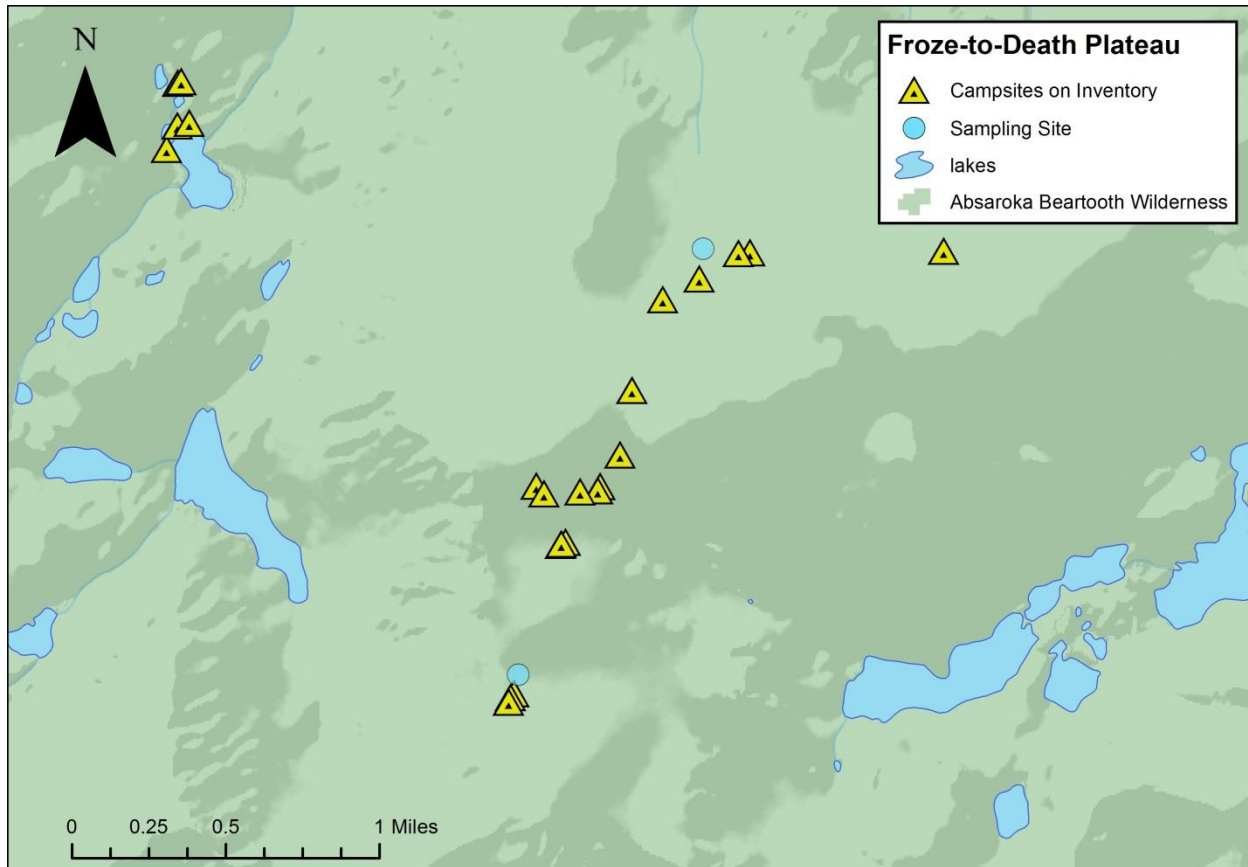


Figure 7: Froze-to-death Plateau, Snowbank 1 to the north and Snowbank 2 to the south.

## Potential improvements to our PCR analysis and research design

Although this was an exploratory analysis to determine if fecal contamination was occurring and if human recreation had an influential role in its occurrence, these results highlight the potential impacts of human recreation on fecal contamination in Wilderness water resources. Our sampling strategy was tailored to determine and quantify fecal bacteria occurrence across the Absaroka Beartooth Wilderness, which was successful in identifying high use areas experiencing human fecal contamination. The next step is to determine the temporal dynamics of recreation derived fecal contamination in Wilderness water sources we detected BacH in by monitoring BacH gene copy levels over time from the preseason(before

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campers are camping) to the postseason(after campers are camping) to accurately quantify the role of recreation on BacH gene copy levels.

An improvement that we could adopt regarding the methods of this study would be to account for variability in size of the water sources in our study. To do this we would collect more samples at sites with larger volumes of water to account for changes in dilution across sites. Our sites in this study ranged from surface areas of over 400 acres (Mystic Lake) to around 4 acres (Ouzel Lake). Although our sites were scaled by dividing the barren core estimates (sq. ft.) by surface area( sq. ft.) in our analysis of recreational impact on total coliform/*E.coli* levels, accounting for this variability in our sampling strategy would a more precise way of accounting for the variability in volume across sites.

Despite our focus on wildlife and human derived fecal bacteria in this study, designated Wilderness areas are also frequented by organisms such as horses, mules, llamas, pack goats and dogs that humans bring into the Wilderness with them. Some Wilderness areas (e.g., the Bob Marshall complex) have extensive trail systems designed for both pack animals and humans and these areas can have substantial impacts from this type of use. Another step managers could take is to look at the impacts of pack stock and pets on fecal bacteria contamination in Wilderness water sources. While humans can be educated on Leave-No-Trace and the effects of recreation on water quality, bacterial contamination by animals must be managed externally by humans. Due to this indirect management of animal waste, areas with high stock use may be more susceptible to fecal bacterial contamination than areas with just human recreation.

Once the correct *Bacteroides* spp. markers are developed for different animal species such as pack stock, hypothetically we could look at gene copies per animal and compare them amongst each other as well as against the universal AllBac to determine relative abundance of each species contribution to total fecal contamination (Paruch et al. 2015). Ideally we would be able to monitor the changes in contribution of each species over time to determine when human recreation is substantially impacting fecal bacteria

levels at different times throughout the main visitation season. Although this may require an intensive sampling strategy which would be logistically challenging considering the location and the seasonal difficulties of getting to the sites, developments in real time PCR may make this possible.

### **Future Research in next generation Microbial Source Tracking**

Dr. Cody Youngbull at the Flathead Lake Biological Station has developed an instant PCR machine what he calls a “DNA tracker” for detecting aquatic invasive species in waterbodies (Taylor 2018). The technology used in Dr. Youngbull’s DNA tracker relies on the same methods used in this study in that he is targeting certain 16s rRNA genes in repeated water sampling to determine the presence/absence of an organism, in his case the zebra mussel, a common aquatic invasive species (Paruch et al. 2015; Taylor 2018; Pendergraph in review). This shoebox sized machine may be used in anybody of freshwater to determine the presence/absence of a particular 16s rRNA gene in a matter of minutes (Taylor 2018). Although the current application for DNA tracker is to detect aquatic invasive species, other primers could possibly be swapped out to determine the presence/absence of other organisms like human derived *Bacteroides*.

The real time PCR results as well as the compact nature of the DNA tracker make it an ideal tool for microbial source tracking of fecal contamination in remote areas like alpine lakes. Many of the sampling specifications like holding times and conditions constrain where we can and cannot sample. By using the DNA tracker, it is just a matter of getting the machine to the sampling site, rather than getting the samples out in time for laboratory analysis. By removing the constraints of holding times and conditions, we would be able to sample more sites more frequently, allowing us to streamline our analysis. Although the DNA tracker is still in development, the results from its test trials are very encouraging and with time this technology should evolve to be cheaper and more accessible for managers (Taylor 2018).



### Conclusion

The results from this study highlight the potential impact of human recreation on water quality. The high occurrence of total coliform and *E. coli* in Wilderness water sources we detected represents a potential public health hazard for visitors pulling water from popular sources adjacent to areas with high recreational use. Validation of human fecal contamination in water sources through microbial source tracking definitively links fecal contamination to human recreation. These results add to an already exhaustive list of recreational impacts from humans to Wilderness water resources and should be concerning for Wilderness managers trying to mitigate impacts to various aspects of Wilderness character (Cilimburg et al., 2000; Marion et al., 2017).

Extensive impacts to popular recreation areas and their natural resources are already determining where and how we recreate in some areas. For example, in the Maroon Bells-Snowmass Wilderness in Colorado, managers have had to result to permitting and restricting numbers of visitors at the popular Conundrum Hot Springs (Aspen Times 2019). Presence of *E.coli* in water resources and substantial impacts to soil and vegetation have forced managers to mitigate these impacts through restricting recreation at this site (CPR 2017). The argument is the natural character of the site has degraded so much that Wilderness managers are forced to confine recreation to help stop further resource degradation and begin restoration to the site. With the increasing impact to the resource, managers may be forced to restrict the places where people recreate in Wilderness, which in itself is as odds with providing opportunities for outstanding and unconfined recreation required by the Wilderness act.

Mitigating human introduced fecal contamination in Wilderness water sources will likely continue to be a problem into the future due to the increased visitation by visitors and the lack of waste facilities provided by agencies. Although Wilderness restrictions on facilities are in place to protect Wilderness character, unrestricted recreational activities may have substantial impacts on Wilderness character,

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particularly affecting the natural and opportunities for unconfined recreation criteria. Passive strategies such as permitting may enable Wilderness managers to somewhat respect the Wilderness Act without taking more invasive measures such as closing certain areas or installations of permanent waste facilities.

The delicate balance of managing for the natural criteria of Wilderness character while still providing opportunities for primitive and unconfined recreation is becoming more and more difficult as the impacts from recreation continue to accumulate. While many Wilderness advocates argue that recreation is an important draw to Wilderness and can be the foundation for future Wilderness advocacy, recreation is having profound impacts on natural resources that visitors and downstream rely on (Wilderness Watch 2019). Opportunities for primitive and unconfined recreation should not translate to tragedy of the commons via a free for all by Wilderness visitors. We as a society could require some restraint regarding Wilderness use and view a permitting system or zoning as providing opportunities for primitive and unconfined recreation while still respecting the natural character of Wilderness. To protect Wilderness Character and water quality managers might consider taking additional steps to prevent fecal water contamination in Wilderness areas while maintaining a rigorous aquatic monitoring program to test the effectiveness of mitigation strategies.

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